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Effect of Clerodane-Type Diterpenoids Isolated from *Salvia* spp. on the Feeding Behaviour of *Spodoptera littoralis*

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Abstract: Twenty-nine clerodane-type diterpenoids isolated from species of *Salvia* growing in Mexico, or analogues of them, were tested for antifeedant activity against *Spodoptera littoralis* larvae using behavioural and electrophysiological bioassays. Eight of the compounds showed potent antifeedant activity in choice and no-choice bioassays and these compounds also stimulated dose-dependent responses from neurones in the lateral styloconic sensilla of *S. littoralis*.

Keywords: antifeedants, neo-clerodanes, *Spodoptera littoralis*, *Salvia*.

1 INTRODUCTION

Some of the neo-clerodanes isolated from plant genera *Teucrium* L., *Ajuga* L. and *Scutellaria* L. (Labiatae) have been shown to have potent antifeedant activity against lepidopteran larvae.[1] To date the most potent of these neo-clerodanes is Jodrellin B isolated from *Scutellaria woronowii* Juz. and *Scutellaria galericulata* L.[2,3] The results of these studies prompted us to evaluate the antifeedant activity of some of the 60 neo-clerodanes that have been isolated during systematic studies of the species of *Salvia* growing in Mexico.[4–15] In this paper we report the effects of 29 of these neo-clerodanes on the feeding behaviour of larvae of *Spodoptera littoralis* (Boisd.) and comment on the structure–activity relationships.

2 MATERIALS AND METHODS

2.1 Compounds

A series of neo-clerodanes were isolated, or derived from compounds isolated, from species of *Salvia* (Labiatae; subgenus Calosphace) growing in Mexico (Fig. 1).[4–15]

2.2 Insects

Larvae of *Spodoptera littoralis*, 24–36 h into the final stadium, were used in both the behavioural and electrophysiological bioassays. Insects were kept at 25(±1)°C under a 16:8 h light:dark photoregime and reared on a wheat-based diet.[16]

2.3 Bioassays

2.3.1 Behaviour

A choice bioassay was used to assess the ability of the insects to perceive the compounds and select between a control disc and a disc treated with a test compound.[17] The compounds were applied to glass-fibre discs (Whatman GF/A 2·1 cm diam.) made palatable by the addition of sucrose solution (50 mM; 100 μl). Control discs carried just sucrose, whereas the treatment discs carried in addition to sucrose, 100 μl of one of the test compounds at one of four concentrations (1, 10, 100 or 500 mg litre^{-1}). The discs were left to dry, and then weighed. In the choice test, larvae were placed individually in Petri dishes (8·5 cm diam.), each with a control

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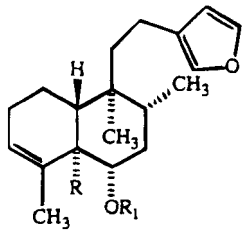
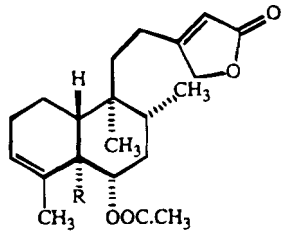
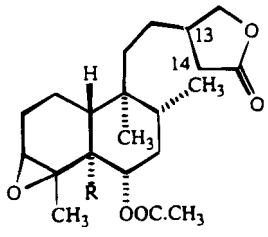
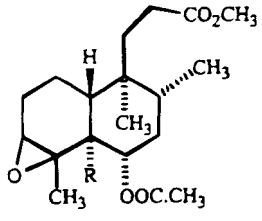
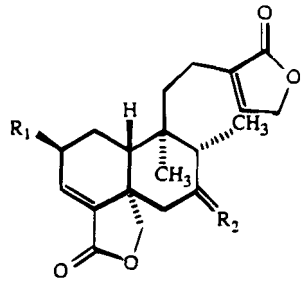
	Compound	Source	Reference
	1. R = CO_2H R_1 = H: Kerlinic acid 2. R = CO_2CH_3 R_1 = CH_3CO: Kerlinic acid methyl ester acetate	*S. keerlii*	4
	3. R = CO_2CH_3: Melissodoric acid methyl ester acetate	*S. melissodora*	5
	4. R = CO_2CH_3: 13,14-dihydro-3,4-epoxy-melissodoric acid methyl ester acetate	*S. melissodora*	5
	5. R = CO_2CH_3: tri-nor-derivative of 3,4-epoxy-melissodoric acid methyl ester acetate	*S. melissodora*	5
	6. R_1 = OOC·CH_3 R_2 = O: 2β-acetoxy-7-keto-*neo*-clerodan-3,13-dien-18,19:16,15-diolide 7. R_1 = OH R_2 = αOOC.CH_3, βH: 7α-acetoxy-2β-hydroxy-*neo*-clerodan-3,13-diene-18,19:16,15-diolide 8. R_1 = OOC·CH_3 R_2 = αOH, βH:2β-acetoxy-7α-hydroxy-*neo*-clerodan-3,13-dien-18,19:16,15-diolide 9. R_1 = H R_2 – αOOC.CH_3, βH: 7α-acetoxy-*neo*-clerodan-3,13-dien-18,19:16,15-diolide 10. R_1 = OH R_2 = αOH,βH: 2β,7α-dihydroxy-*neo*-clerodan-3,13-dien-18,19:16,15-diolide 11. R_1 = OOC.CH_3 R_2 = H,H: 2β-acetoxy-*neo*-clerodan-3,13-dien-18,19:16,15-diolide	*S. melissodora*	6

Fig. 1. Structure of neo-clerodane diterpenoids isolated from different species of *Salvia*.

and treatment disc. The bioassay terminated after 50% of either disc was eaten or after 18 h if the insects had not eaten 50% of either disc. The larvae were removed and the discs dried and reweighed. The antifeedant index $[(C - T)/(C + T)] \times 100$ was calculated, where C and T represent the mass eaten of control and treatment discs, respectively. A positive value for the antifeedant index indicates an antifeedant and a negative value a phagostimulant. The Wilcoxon matched pairs test was used to assess the significance of the activity and probit analysis was used to establish the concentration required to obtain an antifeedant index of 50%

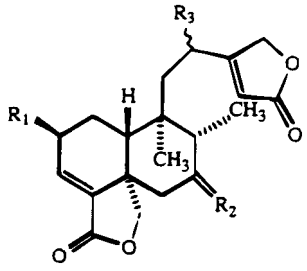
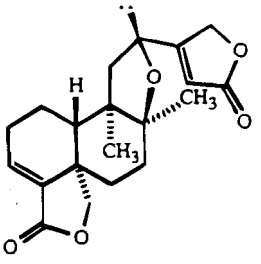
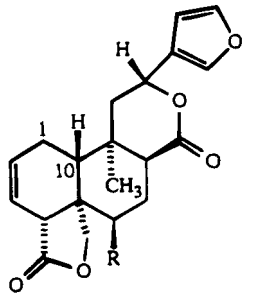
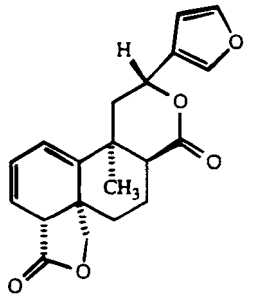
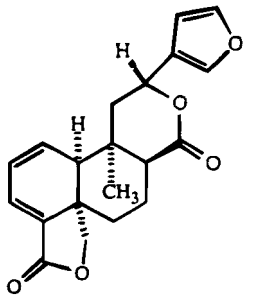
	Compound	Source	Reference
	**12.** $R_1 = R_3 = H$ $R_2 = \alpha OH, \beta H$: 7α-hydroxy-*neo*-clerodan-3,13-dien-18,19:15,16-diolide	*S. semiatrata* *S. melissodora* *S. microphylla* *S. aspera*	7
	13. $R_1 = H$ $R_2 = O$ $R_3 = H$: 7-keto-*neo*-clerodan-3,13-dien-18,19:15,16-diolide	*S. aspera*	
	14. $R_1 = OH$ $R_2 = H,H$ $R_3 = OH$ (12*S*): Semiatrin	*S. semiatrata*	7
	15. $R_1 = H$ $R_2 = \alpha OOC.CH_3$, βH $R_3 = OH$(12*R*): Kerlinolide	*S. keerlii*	8
	**16.** Kerlin	*S. keerlii*	8
	**17.** R = H: Salviarin **18.** R = OH: 6β-hydroxysalviarin	*S. rhyacophilla*	9
	**19.** 1(10)-dehydrosalviarin	*S. lineata*	10
	**20.** Linearolactone	*S. lineata*	10

Fig. 1.—(continued)

(AI_{50}). There were 10 replicates per concentration of each compound.

A no-choice bioassay was used to test whether the compounds could suppress feeding for a period of time. The discs were prepared as in the choice bioassay. Only one concentration (100 mg litre^{-1}) of the test compounds was used. Larvae were exposed to a single, control or treatment disc. Experiments were undertaken

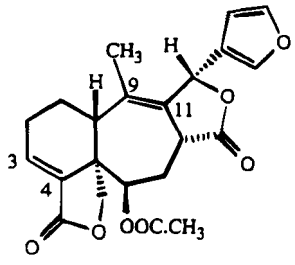
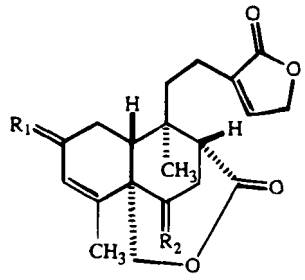
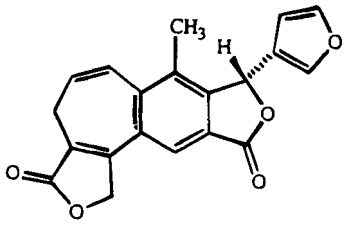
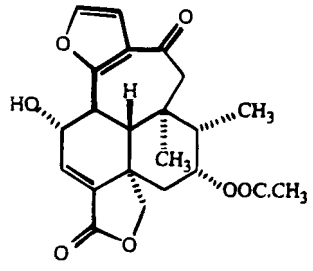
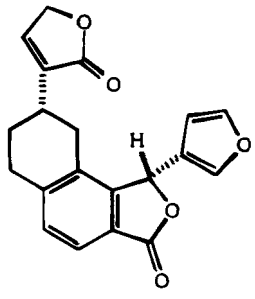
	Compound	Source	Reference
	**21.** Salvigenolide **22.** 3,4β-dihydro: dihydrosalvigenolide **23.** 9β,11β-epoxy: epoxysalivigenolide	*S. fulgens*	11
	**24.** R_1 = H,H R_2 = αOH,βH: salvimadrensin **25.** R_1 = H,H R_2 = O: dehydro-salvimadrensin **26.** R_1 = O R_2 = H,H: salvimadrensinone	*S. madrensis*	12
	**27.** Isosalvipuberuline	*S. puberula*	13
	**28.** Salvisousolide	*S. urolepis* *S. sousae*	14
	**29.** Tilifodiolide	*S. tiliaefolia*	15

Fig. 1.—(continued)

in blocks of nine or 10 different compounds and a control, with five replications per compound in each block. Each compound was tested in two blocks to give a total of 10 replicates per compound. Bioassays within a block were terminated when the larvae exposed to the control discs had eaten approximately 50% of the control discs. The larvae were then removed and the discs dried and reweighed. The Mann–Whitney test was used to compare the amounts eaten of the treatment and control discs.

2.3.3 *Electrophysiology*

The lateral and medial maxillary styloconic taste sensilla of final stadium larvae of *S. littoralis* were stimulated with solutions of each compound dissolved in the

TABLE 1
Behavioural and Electrophysiological Responses of *Spodoptera littoralis* Larvae to the Neo-clerodanes

	Behaviour					*Electrophysiology*			
	Choice bioassay Antifeedant index			*No-choice bioassay Amount eaten (%)*		*Lateral sensillum impulses in first sec.*			
Compound	*Mean*[a]	*SEM*	AI_{50}[b]	*Mean*[c]	*SEM*	*Mean*[d]	*SEM*	R^2[e]	*Slope*[f]
1	−1	12·8	>1000	44	7·1	10	2·3	0·342	
2	−8	10·2	>1000	51	2·1	8	3·5	0·321	
3	−11	9·8	>1000	48	3·5	11	3·8	0·273	
4	73*	5·2	1	15*	3·2	45	2·3	0·966*	21·7
5	18	4·8	>1000	29	11·8	21	4·5	0·274	
6	−15	11·1	>1000	51	6·2	8	2·9	0·276	
7	54*	15·4	122	22	9·9	45	4·3	0·769*	9·4
8	83*	8·3	84	7*	1·6	44	5·8	0·744*	15·6
9	29*	5·3	192	28	9·5	35	3·2	0·956*	14·5
10	34*	4·6	142	26	9·4	39	3·8	0·992*	11·2
11	6	2·5	>1000	48	4·1	10	3·9	0·165	
12	32*	14·5	139	26	9·5	36	2·9	0·922*	12·3
13	26*	6·8	294	34	3·4	41	3·5	0·959*	13·6
14	57*	6·7	87	12*	1·8	39	2·9	0·963*	12·7
15	70*	10·3	67	7*	3·2	41	2·9	0·843*	14·0
16	7	21·2	>1000	29	9·1	11	4·3	0·165	
17	59*	5·2	81	18*	2·1	38	3·9	0·961*	11·8
18	66*	8·3	24	7*	1·8	48	2·9	0·747*	12·0
19	66*	8·4	32	15*	1·4	41	2·6	0·627*	9·5
20	18	8·9	>1000	51	2·6	18	4·6	0·175	
21	11	2·4	>1000	48	5·7	11	4·8	0·264	
22	20	13·3	>1000	46	10·5	13	3·8	0·176	
23	−7	3·1	>1000	41	4·8	8	2·8	0·105	
24	27*	10·2	194	30	9·8	45	2·9	0·851*	13·1
25	20	4·5	254	27	9·4	29	2·9	0·176	
26	3	7·1	>1000	45	7·9	8	2·7	0·184	
27	39*	12·9	396	43	5·6	16	3·9	0·048	
28	13	1·5	>1000	55	2·1	21	3·4	0·175	
29	51*	10·4	91	16*	4·1	39	2·9	0·848*	12·8
Control				51[g]	2·1	13[h]	2·8		

[a] Mean antifeedant index $((C - T)/(C + T))\%$ at 100 mg litre^{-1}, * Antifeedant activity significant at $P < 0{\cdot}05$, Wilcoxon matched pairs test.
[b] AI_{50} is the concentration of the compound that gives an estimated antifeedant index of 50% (probit analysis).
[c] * Differs significantly from control at $P < 0{\cdot}05$, Mann-Whitney test.
[d] Mean number of action potentials in the first second of stimulation with a compound at 1 mM.
[e] Dose-dependent response, R^2 regression analysis, * significant difference at $P < 0{\cdot}01$.
[f] Slope of regression line.
[g] Mean amount eaten by larvae of sucrose-treated control discs.
[h] Mean number of action potentials in the first second of stimulation with the electrolyte potassium chloride (5 mM).

electrolyte, potassium chloride (50 mM).[17] Every compound was tested at five different concentrations (0·1, 1, 5, 10 and 50 mM). Each sensillum was first stimulated with the electrolyte for 1 s then with one to three of the test compounds, each at sequentially increasing concentrations. The successive 1-s stimulations of each sensillum were separated by at least 2 min. The responses of a sensillum to a compound were measured in terms of the total number of action potentials in the first second of stimulation. Due to the limited availability of the compounds the number of larvae stimulated with the different concentrations of each compound was either five (10 and 50 mM) or 10 (0·1, 1 and 5 mM).

3 RESULTS AND DISCUSSION

Analysis of the data from the choice bioassay shows that 15 of the 29 compounds tested has significant antifeedant activity at 100 mg litre^{-1} (Table 1). Eight of

these 15 compounds (**4**, **8**, **14**, **15**, **17**, **18**, **19** and **29**) had AI_{50} values of less than 100 mg litre^{-1} and can be considered as potent antifeedants (Table 1). These same eight compounds were the only compounds to show significant antifeedant activity, when tested at 100 mg litre^{-1} in the no-choice bioassays (Table 1).

All compounds that showed significant activity in the behavioural bioassays, with the exception of compound **27**, stimulated dose-dependent responses from neurones in the lateral styloconic sensilla (Table 1), whereas none of the 29 compounds stimulated dose-dependent responses from neurones in the medial styloconic sensilla. The responses from the medial sensilla varies from 4($\pm$1·6) to 12($\pm$2·1) impulses in the first second of stimulation. The responses from the lateral sensilla correlated with the antifeedant activity of the compounds (Choice bioassay, R^2 0·736, $P < 0{\cdot}001$: No-choice bioassay, R^2 0·73, $P < 0{\cdot}001$), suggesting that, in these experiments, the neural input from the lateral sensilla could be an important determinant of the resulting behaviour response. A similar relationship between neural input and behaviour was shown earlier for a range of clerodanes.[18] However, in the earlier study the compounds stimulated responses from at least one neurone in the medial sensillum. In that study, the activity of the compounds was thought to be associated with the presence of a furan side chain, acetate substitution at C6 and C19 and an epoxide group at C4–C18. In the present study the active compounds have terminal furan and α- or β-butenolide groups, functionalities also present in many of the less active compounds, and none of the compounds have an epoxide at C4–C18 (Fig. 1).

Consideration of the antifeedant activity of the compounds in relation to their structure enables us to evaluate structure–activity relationships. For example, comparison of the responses of *S. littoralis* to **2** and **3** indicates that altering the composition of the C9 side chain from a β-substituted furan ring (**1** and **2**) to a β-butenolide function (**3**) did not alter activity. Treatment of **3** with *m*-chloroperbenzoic acid resulted in the formation of a C3–C4 epoxide and a compound (**4**) with potent antifeedant activity (Table 1). However, further oxidation of **4** and esterification resulted in the formation of a dimethyl ester (**5**) and a loss of antifeedant activity.

Compounds **6–23** and compound **28** contain a C18–C19 lactone (Fig. 1). The configuration of this lactone and the composition of substituents on the decalin ring differ among the compounds. Compounds **6–16**, **20–23** and **28** contain an α-, β-unsaturated-18,19 olide function and some of them (**7–10**, **12**, **14**, **15** and **29**) showed antifeedant activity in the choice bioassay, whereas all three compounds (**17–19**) that have a saturated C18–C19 olide function showed significant levels of activity (Table 1). This contrasts with other studies which have shown that the presence of a saturated C18–C19 olide system resulted in a loss of antifeedant activity.[19]

Compounds **6–11** vary in the composition of the functional groups present at C2 and C7, those compounds with either an acetoxy or hydroxy group at C7 showing potent antifeedant activity in the choice bioassay (**7–10**). Compound **8**, with an hydroxy at C7 and acetoxy at C2, was the only compound in this group to show potent activity in the no-choice bioassay (Table 1).

Compounds **14** and **15** showed significant antifeedant activity in both behavioural bioassays. These two compounds have a hydroxy group at C12 which differs between compounds in its configuration. Semiatrin (**14**) contains the *S* configuration, whereas kerlinolide (**15**) contains the more common *R* configuration. However, other substitutions on these two molecules differ, so it is not possible to determine the exact importance of the stereochemistry of the C12 hydroxy for the activity of the compound. Compound **27** is the only one of the five compounds which contains a seven-carbon ring (**21–23**, **27** and **28**) to show significant antifeedant activity (choice test only). This compound did elicit a response from the lateral sensillum but unlike the other compounds showing antifeedant activity this response was not dose-dependent, suggesting a difference in the ligand binding properties of this compound. This ring formation is not commonly reported in neo-clerodanes.[12]

Another compound with an unusual combination of functionalities is tilifodiolide (**29**), isolated from *S. tiliaefolia*.[15] It contains an α-substituted butenolide function, a β-substituted furan ring, a C12–C17 γ-lactone group and a tetra-substituted aromatic ring (Fig. 1). This compound showed potent antifeedant activity in both of the behavioural bioassays (Table 1).

4 CONCLUSIONS

We have shown that neo-clerodanes with a range of different substitutions show significant antifeedant activity and that these compounds elicit dose-dependent neural responses from taste sensilla on the mouthparts of larvae of *S. littoralis*. Although these neural responses correlate with the behavioural responses it does not mean that the neural response is the sole determinant of the behavioural response, but it does suggest a causal relationship. Thus these bioassays can be used to study how small changes in the moieties in the decalin and/or side chain of the compounds can influence their potency as antifeedants. This study has shown that functional groups previously shown to be associated with the insect antifeedant activity of neo-clerodanes are not essential for that activity.[1,19,20] For example, not all of the compounds present in this study have a furan side chain nor do they have acetate substitutes at C6 and

C19 or an epoxide at C4–C18. As yet no substitution pattern can be identified as essential for the antifeedant activity of the clerodane-type compounds against *S. littoralis*. Further research is needed to identify the functional groups, or combinations of groups, on these molecules reponsible for their potency and to determine whether these compounds will deter other insects from feeding and whether they influence the behaviour of beneficial insects, such as parasitoids or predators.

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